The Benefit of Interferon-Gamma Release Assay for Diagnosis of Extrapulmonary Tuberculosis

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ABSTRAK

Latar belakang: *sudah banyak penelitian mengenai interferon-gamma release assay (IGRA) dalam TB luar paru, namun hanya sedikit yang berasal dari negara-negara berkembang. Ini merupakan penelitian pertama tentang kegunaan IGRA dalam TB luar paru yang dilakukan di Indonesia sebagai negara berkembang dengan kasus TB terbanyak kedua di dunia. Studi ini bertujuan untuk mengetahui manfaat pemeriksaan IGRA dalam mendiagnosis TB ekstraparu.* ***Metode:*** *sebanyak 84 pasien dengan dugaan TB ekstraparu dilakukan pemeriksaan IGRA dan pemeriksaan baku emas secara tersamar. Pemeriksaan baku emas dilakukan pemeriksaan histopatologi dan pewarnaan BTA jaringan.* ***Hasil:*** *dari total 84 pasien didapatkan hasil baku emas positif pada 57 pasien, dimana 50 pasien diantaranya didapatkan hasil IGRA positif. Dari 27 pasien dengan baku emas negatif didapatkan hasil IGRA positif pada 10 pasien. Limfadenitis TB merupakan manifestasi TB ekstraparu yang paling banyak ditemukan. Hasil uji diagnostik IGRA untuk TB ekstraparu yang didapat adalah sebagai berikut: sensitifitas 87,71%, spesifisitas 63%, nilai duga positif 83,33%, dan nilai duga negatif 70,83%.* ***Kesimpulan:*** *pemeriksaan IGRA dapat digunakan sebagai salah satu sarana penunjang diagnosis TB ekstraparu, namun hasil yang negatif belum dapat menyingkirkan kemungkinan adanya infeksi TB tersebut.*

Kata kunci: *TB ekstraparu, IGRA, histopatologi, BTA jaringan.*

ABSTRACT

Background: *there are many researches about IGRA in extrapulmonary Tuberculosis (TB), but there only few data from developing countries. This was the first research about the utility of IGRA in extrapulmonary TB performed in Indonesia as developing country with the 2nd most frequent of TB cases in the world. This study aimed to identify the advantage of IGRA examination in diagnosing extrapulmonary TB.* ***Methods:*** *eighty-four patients, presumed to have extrapulmonary TB were examined with IGRA and gold standard examination. The gold standard examination was performed by histopathologic examination, and tissue smear for acid-fast bacilli.* ***Results:*** *among 84 patients included in the study, 57 patients were tested positive with gold standard, where 50 patients among them were also tested positive with IGRA. Among 27 patients tested negative with gold standard, IGRA positive was found in 10 patients. Lymphadenitis was the most common manifestation of the extrapulmonary TB. Diagnostic test from IGRA for extrapulmonary TB found as follows: sensitivity 87,71%, specificity 63%, positive predictive value 83,33%, and negative predictive value 70,83%.* ***Conclusion:*** *IGRA could be used as supporting tool in the diagnosis of extrapulmonary TB. The negative result, however, does not indicate absence of TB infection.*

Keywords: *extrapulmonary TB, IGRA, histopathology, tissue acid-fast bacilli.*

INTRODUCTION

Tuberculosis (TB) is still a global health problem. In 2016, it was estimated that there were 1.3 million deaths due to TB in non-HIV patients and 374.000 deaths in HIV patients. It was also estimated that there were 10.4 million TB patients in 2016, and 56% came from 5 countries with the most-TB cases in the world, namely India, Indonesia, China, Philippines, and Pakistan.[1] Although most of the patients suffered from pulmonary TB, about 15-25% suffered from extrapulmonary TB. Of all extrapulmonary TB patients, most cases commonly found were lymph node TB (35-40%), followed by pleural TB (20-30%), bone and joint TB (5-10%), genitourinary TB (3-6.5%), tuberculous meningitis (5-6%), peritoneal TB (3%), and others (11.8%).[2-6]

Extrapulmonary TB is usually difficult to diagnose due to the unspecific symptoms, while the acid-fast bacilli smear and culture from tissue and body fluid often reveal negative result. Hence, an invasive procedure is usually required for histopathologic diagnosis.[7,8] The International Standard for TB Care 2014 also suggested Gene-Xpert MTB/RIF tissue examination to diagnose extrapulmonary TB. However such examination is still not feasible.[7] The measurement of adenosine deaminase (ADA) level in body fluid is usually used as an alternative, although the utilization is still limited to pleural TB, pericardial TB and tuberculous meningitis.[9] Furthermore ADA level within the body fluid will also increase due to purulent bacteria infection, SLE or lymphoproliferative diseases.[3]

On the other hand, immunoassay staining to detect Mycobacterium tuberculosis-specific immune host response is becoming popular as alternative tools for extrapulmonary TB diagnosis. Mycobacterium tuberculosis (M.TB) will initiate cascade of immune respons which triggers cytokines secretion as well as Th1 lymphocyte activation. The level of interferon-γ (IFN- γ) as one of the cytokines produced by Th1 cell, will increase due to M.TB infection. The Interferon-Gamma Release Assay (IGRA) aim to measure the level of IFN-γ released within the blood after being stimulated by purified protein derivate obtained from M.TB. Interferon-Gamma Release Assay in QuantiFERON Gold-In Tube assay (QFT-GIT) used 3 M.TB-specific antigens, namely early secreted antigenic target 6 (ESAT-6), culture filtrate protein 10 (CFP 10) and TB7.7 that only found in M.TB and not in either BCG strain or other nontuberculous mycobacteria.9 The goal of this study is to investigate the benefit of IGRA with ELISA-based QuantiFERON-TB Gold In-Tube assay to diagnose extrapulmonary TB. There are many researches about IGRA in extrapulmonary TB, but most of them come from high-income countries and only a few from middle/low-income countries. This is the first research about the utility of IGRA in the diagnosis of extrapulmonary TB performed in Indonesia as developing country with the 2nd most frequent of TB cases in the world.

METHODS

This cross-sectional study was done in Cipto Mangunkusumo hospital. Samples were collected from October 2015-October 2017. This study had been approved by ethical clearance from the ethics committee Faculty of Medicine Universitas Indonesia on November 9th, 2015, with a reference number 976/UN2.F1/ETIK/2015.

Patients Selection

Samples were collected using consecutive methods, to include all patients suspected with extrapulmonary TB who came to the Pulmonology clinic and was admitted to Cipto Mangunkusumo Hospital. The inclusion criteria include over 18 year old patients, suspected with extrapulmonary TB but were not under any anti-tuberculosis medication, and consented to be subject of this study. The exclusion criteria include extrapulmonary TB patients with a homeostatic disturbance which prevents invasive intervention for tissue specimen collection. All patients who met the inclusion criteria underwent anamnesis, physical examination, routine laboratory, and radiologic examination according to the organ involvement. After that with blinding design IGRA examination from a blood specimen was performed to be compared with either histopathologic or tissue smear for acid-fast bacilli (AFB) as the gold standard. To obtain tissue specimen, a biopsy was performed through invasive intervention from the organs

involved. A positive result from biopsy indicates the presence of datia Langhans cell, epithelioid cell, granuloma with central caseous necrosis or AFB in the tissue.

ELISA-based QuantiFERON-TB Gold In-Tube Assay

Three ml blood sample was drawn and stored into 3 different tubes, namely Nil tube, TB antigen tube and mitogen tube. Tuberculosis antigen tube contains 3 kinds of TB-specific antigen coated on its wall namely ESAT-6, CFP-10, and RB7.7. The TB antigen tube was used to assess the IFN-γ production by the T- lymphocyte in response to the TB-antigen. Mitogen tube contains phytohemagglutinin that could trigger lymphocyte cell proliferation, indicating the presence of a viable cell. After 1 ml blood sample was obtained, all tubes were shaken slowly for 5 seconds until the blood sample covered the inner layer of the tube wall. This step was performed to optimize the interaction between blood sample with the TB-antigens on the tube wall. All blood samples were sent to the laboratory to be incubated at 37^0 C for 16-24 hours. After the incubation, all tubes were centrifuged in 2000-3000 g (RCF) for 15 minutes. Two hundred µL blood plasma was pipetted from the tube and added to the Quantiferon ELISA plate. Then, IGRA assay with ELISA was performed using the automated ELISA workstation. Data from IGRA were analyzed using Quaniferon Analysis Software.[8]

Interpretation

Results obtained from IGRA were reported qualitatively in 3 categories: positive, negative and indeterminate. A positive result was indicated by the difference between IFN-γ level in the TB antigen tube and in the Nil tube of ≥ 0.35 IU. To control for the presence of non-specific IFN-γ, the response in the TB-antigen tube must be more than 25% of the level of IFN-γ in the nil tube. The indeterminate result was indicated by no increase in the level of IFN-γ in the mitogen tube or when there was a high level of non-specific-IFN-γ in the nil tube (>8 IU). Therefore re-examination was performed in indeterminate result. By implementing positive internal control (mitogen tube), the IGRA assay could differentiate indeterminate result from the true negative result.[8]

Data Analysis

The data analysis was done using SPSS 20. Descriptive data were presented as the proportion of extrapulmonary TB and its clinical manifestation. The data were analyzed using 2x2 chi-square test to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) with 95% confidence interval (CI).

RESULTS

Out of 84 patients suspected with extrapulmonary TB with median age of 33.5 years (range 17-83 years), 57 patients were confirmed to have extrapulmonary TB based on gold standard examination. The characteristic of the subject and the type of extrapulmonary TB is shown in **Table 1**.

Table 1. Subject characteristic

Characteristic	Total (N=84)
Age (years), median (range)	33.5 (17-83)
Gender (Male), n (%)	33 (39.3)
Positive gold standard, n (%)	
- Histopathology (+)	56 (66.0)
- Tissue smear for AFB (+)	1 (1.19)
Negative gold standard, n (%)	27 (32.1)
Extrapulmonary TB Type, n (%)	
- Lymph node TB	39 (46.2)
- Cutaneus TB	9 (10.7)
- Tuberculous spondylitis	3 (3.57)
- Peritoneal TB	2 (2.38)
- Tuberculous meningitis and lymph node TB	1 (1.19)
- Tuberculous hepatitis	1 (1.19)
- Genital TB	1 (1.19)
- Renal TB	1 (1.19)

Most of the extrapulmonary TB patients were diagnosed based on histopathologic examination, with lymph node TB as the most frequent clinical manifestation. Twenty seventh patients were tested negative for extrapulmonary TB with gold standard examination. Most of them suffered from chronic non-specific lymphadenitis and metastatic lymph enlargement as shown in **Table 2**.

Table 2. Another extrapulmonary disease

Involved Organ	N=27
Lymph, n (%)	
- Chronic non-specific lymphadenitis	10 (37.0)
- Metastatic lymph enlargement	2 (7.40)
- Kikuchi Fujimoto lymphadenitis	1 (3.70)
- Epidermoid cyst	1 (3.70)
- Sialadenosis	1(3.70)
Skin, n (%)	
- Hyperplasia epitheliomatosis	1 (3.70)
- Actinomycosis	1 (3.70)
- Achantocys epidermis	1 (3.70)
- Chronic Ulcer	1 (3.70)
- Epidermis cyst	1 (3.70)
Bone, n (%)	
- Chronic non-specific spondylitis	2 (7.40)
- Chronic non-specific osteomyelitis	2 (7.40)
- Others, n (%)	
- Chronic non-specific mastitis	2 (7.40)
- Liver Cirrhosis	1 (3.70)

Table 3 shows sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio from IGRA compared to the gold standard.

Table 3. Cross-tabulation of IGRA and gold standard

IGRA	Gold standard		Total
	Positive	Negative	
Positive	50	10	60
Negative	7	17	24
Total	57	27	84

In this study, the sensitivity value of IGRA was 87.71% (95% CI 77.0-94,0%), while the specificity was 63.0% (95% CI 44.0-78%). Positive predictive value of IGRA was 83.33% (95% CI 72.0-91.0%), while negative predictive value was 70.83% (95% CI 51.0-85.0%). Positive likelihood ratio of IGRA was 2.37 (1.39-4.02), while the negative likelihood ratio was 0.2 (0.09-0.41).

DISCUSSION

Among 84 patients suspected with extrapulmonary TB, 57 were definitively diagnosed with extrapulmonary Tb based on gold standard with sensitivity and specificity of 87.7% (95% CI 77.0-94%) and 63.0% (95% CI 44.0-78%), respectively. Yun Feng et al.[10] found a better sensitivity and specificity values compared with our study namely 93.3% (95% CI 77.9- 99.2%) and 88.9% (95% CI 80-94.8%). However, in that study extrapulmonary TB was not entirely diagnosed based on the gold standard. In some patients, the presence of extrapulmonary TB was diagnosed only based on clinical symptoms and radiological features, thus affecting the validity of the study. Furthermore clinical manifestations of extrapulmonary TB in that study showed a different spectrum from our study; most of the patients (40%) exhibited central nervous system involvement, while lymph node involvement was only found in 10% of patients.[10] In our study, lymph node TB was found in the majority of patients (46%), whereas tuberculous meningitis was only found in 1% of the patients.

The study from Oh-Hyung Cho et al.[11] found a similar sensitivity value with our research, that is 84% (95% CI 78-89%), but with a lower specificity of 51% (95% CI 43- 58%). In that study, out of 153 patients (77.78%) were diagnosed with extrapulmonary TB based on gold standard, while the remaining 34 patients (22.22%) were diagnosed only based on clinical symptoms and response to anti-tuberculosis treatment. Moreover, the indeterminate results of IGRA examination which was found in 8 patients (5.2%) were directly included to the group with negative IGRA outcomes.[11] Both of these would have affected research validity because in addition to the diagnosis problem that was not entirely accurate, the results of indeterminate IGRA was not necessarily negative. Indeterminate IGRA result means that the result cannot be concluded as negative or positive, which may result from errors in examination technique or may be due to severe immune deficiency.[12,13] In severe immune deficiency there will be a significant decrease in CD4 cell count so that the production of IFN-γ will also be greatly decreased. To determine, it should be re-examined with better examination technique. In our study, from 84 patients examined by IGRA, no indeterminate outcome was found.

Lin Fan et al.[14] conducted a meta-analysis study with two different IGRA examination

techniques, namely the ELISA-based QFT-GIT and ELISpot-based T-SPOT.TB. In ELISpot-based T-SPOT.TB the number of T cells that produce IFN-γ were calculated after exposure with all three antigens as in QFT-GIT. The QFT-GIT pooled sensitivity and specificity were 72% (95% CI 65-79%) and 82% (95% CI 78-87%), respectively. T-SPOT.TB showed a better pooled sensitivity value of 90% (95% CI 86-93%), but a lower pooled specificity of 68% (95% CI 64-73%). In this study, subgroup analysis was performed based on patient's economic level. Sensitivity and specificity values were still good enough in high-income countries namely 79-89% and 73-83%, respectively; but in low/middle-income countries, lower values were obtained (29% with QFT-GIT and 34% with T-SPOT.TB).[14] There were some limitations in that meta-analysis, because 17 from 20 selected original studies were from high-income countries and only 3 studies from low/middle-income countries.[14] Also, although the clinical manifestations of extrapulmonary TB in that study included a variety of organ involvement, research on low/middle-income countries. Involved only one organ system, each of which is pleura and meninges. This is different from our study that showed involvement of various organ systems with the most frequent manifestation being lymph node TB. The sensitivity of IGRA was influenced by antigen levels, host response to its antigen and the manifestations of organ involvement. With higher antigen level that marked by high levels of ESAT-6, CFP-10, and TB7.7 produced by genes in the region of difference-1 (RD1), the formed immune response will be stronger. In lymph node TB, high levels of antigen are obtained with a good immune response so that lymphocyte cells will produce IFN-γ efficiently. In contrast to tuberculous meningitis, low levels of antigen are found with low to moderate levels of host immune responses so that IFN-γ production by lymphocytes also becomes lower.[11] This may explain why in this meta-analysis, when subgroup analysis is obtained, low sensitivity and specificity values were found in low/middle-income countries. In addition, blinding design which is a required in diagnostic research was only performed in 5 original research.

Shin JA et al.[15] obtained a lower sensitivity value than our study of 70.2% (95% CI 63.7-74.8%) with a similar specificity of 66.7% (95% CI 32.9-90.6%). In subgroup analysis based on the organs involvement, sensitivity and specificity of IGRA in lymph node TB were better namely 81.8% (95% CI 61.4-90.4%) and 80% (95% CI 35.1-98.9%). Contrastingly, pleural TB has lower values of 38.5% (95% CI 31.25-45.7%) and 50% (95% CI 2.7- 97.3%).[15] Based on this study, IGRA in extrapulmonary TB is particularly useful in diagnosing lymph node TB compared with tuberculosis that affect other extrapulmonary organs.[15]

Adilistya et al.[16] conducted a study using IGRA on pleural fluid with T-SPOT.TB to diagnose pleural TB. Sensitivity and specificity value from that study were 100% (95% CI 97-100%) and 88.89% (95% CI 51.75-99.72%), with PPV and NPV of 97.5% (95% CI 86.84-99.94%) and 100% (95% CI 63-100%), respectively. Although IGRA's diagnostic performance was much better than our study, there was a limitation that could affect study validity. This limitation was related to cut-off value for IGRA positivity in pleural fluid. In that study, IGRA test on pleural fluid was considered positive when there was ≥ 6 spot-forming units (SFU), which described the number of active T cells producing IFN-γ.[16] Until now there is no standard IGRA cut-off value for T-SPOT.TB in pleural fluid. Xiao-Xia Zhou et al.[17] in their meta-analysis also obtained a good pooled sensitivity and specificity of T-SPOT.TB for pleural liquid, 92% (95% CI 88-95%) and 85% (95% CI 78-91%), without using standard IGRA cut-off value. The current cut-off value for IGRA in pleural fluid is provided only for QFT-GIT, which varies in value from 0.3 U/L to 10 U/L.[8]

Other meta-analysis studies were also performed by Qianqian Liu et al.[18] that were conducted in patients suspected with lymph node TB. From 10 selected original research, the pooled sensitivity and pooled specificity were 89% (95% CI 85-92%) and 81% (95% CI 77-83%), respectively. Extrapulmonary TB diagnosis in this meta-analysis were also not entirely based on the gold standard. In some

patients, the diagnostic criteria were based on the clinical response to anti-tuberculosis treatment.[18] Although that meta-analysis study shows similar sensitivity to our research and even with better specificity values, since it only involved one organ, it was less likely to describe extrapulmonary TB clinical spectrum in daily practice.

There is a limitation in our study. From 84 patients enrolled, none of them showed any pleural involvement. This is because for diagnosing pleural TB there is currently available a safer and less invasive diagnosis modalities, with a good sensitivity and specificity namely ADA examination. Adenosine deaminase cut-off value for pleural TB is > 40 U/L with sensitivity and specificity of 89-99% and 88-97%, respectively.[8] The availability of ADA examination results in a pleural biopsy performed by pleuroscopy becomes overly invasive, requiring more expensive costs, and a higher risk of intervention.

CONCLUSION

The IGRA examination that is used to diagnose extrapulmonary TB shows good sensitivity and PPV of 87.7% and 83.3%, respectively, therefore IGRA examination can be used as a supporting tool for diagnosis. A low specificity value (63%) indicates that negative IGRA results cannot exclude possibilities of extrapulmonary TB. Other modalities are still needed to confirm presenting of extrapulmonary TB with either non-invaive or invasive measure.

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